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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
TAKESHI DOI, ET AL.	: EXAMINER: O'DELL, DAVID K.
SERIAL NO: 10/574,972	:
FILED: APRIL 7, 2006	: GROUP ART UNIT: 1625
FOR: CYCLIC AMINE ANGIOGENESIS INHIBITORS	:

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Now comes Takeshi Doi who states that:

1. I am a named inventor of the above-identified application.

2. I have been employed by Kowa Company, Ltd. for 26 years as a scientific researcher in the field of Life Science.

3. I understand the English language, or at least the contents of the Declaration were made clear to me prior to executing the same.

4. The following experiment was carried out by me or under my direct supervision and control.

Human umbilical vein endothelial cells (HUVEC) were added to a 96-well plate (1×10^4 cells/well). On the following day, vascular endothelial growth factor-A (VEGF-A) (10 ng/mL) and Compound 1 (i.e., 4-[N-(4-methoxyphenyl)-N-[[5-(3,4,5-trimethoxyphenyl)pyridin-3-yl]methyl]amino]-1-[[2-(3,4,5-trimethoxyphenyl)pyridin-4-yl]methyl]piperidine citrate) of the present invention were added to each of the

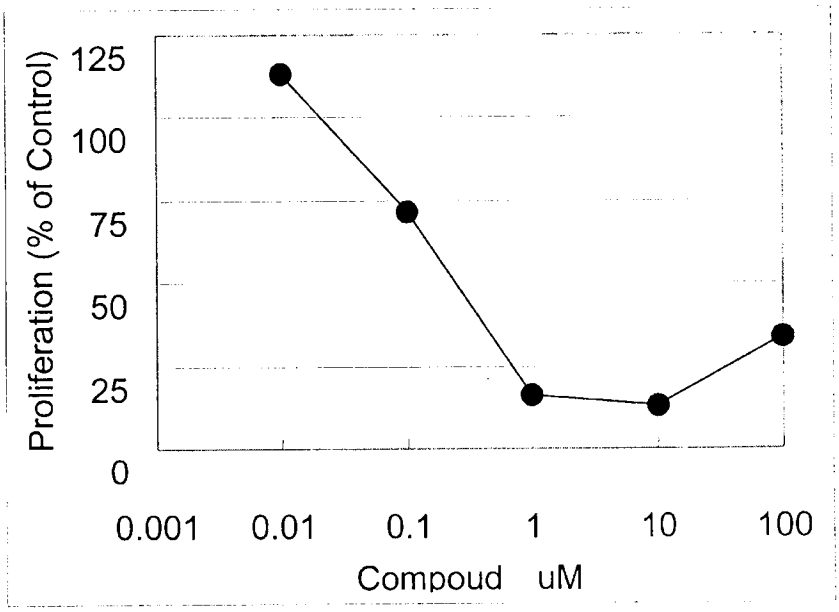
wells. After culturing for 48 hours, the level of proliferation was determined by a colorimetrical determination method using a WST-1 Cell Proliferation Reagent. The determination was carried out three times at each concentration of Compound 1, and proliferation (%) was calculated from the following formula by using the average value of three-time determinations.

Formula

Proliferation (%) = {(OD value at 48 hours after adding Compound 1) – (OD value at 0 hour) / (OD value at 48hours after (control group)) – (OD value at 0 hour)} × 100

5. Experimental Results

Figure A (Inhibiting Effect of Compound 1 against VEGF-A and the proliferation of HUVEC



6. As shown in Figure A above, Compound 1 of the present invention surprisingly exhibits a potent inhibitory effect on VEGF-A and the proliferation of human umbilical vein endothelial cells.

7. Although some compounds may inhibit angiogenesis by exhibiting inhibitory effects on endothelial cell adhesion, not all angiogenesis inhibitors exhibit inhibitory effects on endothelial cell

adhesion. For example, maspin is a *protease inhibitor* that inhibits angiogenesis by exhibiting inhibitory effects on endothelial cell *migration*; vasostatin, calreticulin, prothrombin and antithrobin III are angiogenesis inhibitors that exhibit inhibitory effects on endothelial cell *proliferation*; while angiostatin and VEGI are angiogenesis inhibitors that induce *apoptosis* of endothelial cells.

8. Not all angiogenesis inhibitors that exhibit inhibitory effects on vascular endothelial growth factor (VEGF) necessarily exhibit inhibitory effects on endothelial cell adhesion. For example, VEGF-Trap is a decoy receptor substance that inhibits angiogenesis by binding to and inactivating VEGF; bevacizumab is a monoclonal antibody that inhibits angiogenesis by binding to and inactivating VEGF; and sunitinib is a VEGF receptor tyrosine kinase (RTK) inhibitor that inhibits angiogenesis by disrupting VEGF receptor-mediated signaling. However, it is unknown whether these anti-VEGF angiogenesis inhibitors exhibit inhibitory effects on endothelial cell adhesion.

9. Therefore, it would not have been obvious to a skilled artisan that the cyclic amine compounds represented by general formula (1) which exhibit inhibitory effects on endothelial cell adhesion would exhibit an inhibitory effect on VEGF-A.

10. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Takeshi Doi
Signature

Feb. 24. 2009
Date